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One Health & Zoonotic Threats: An Integrated Approach and 3rd Annual Meeting of the AL4AnimalS Thematic Line Emergent Infectious Diseases and Zoonoses

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INVITED SPEAKERS

Beatriz Romero is an Associate Professor of the Animal Health Department of the Veterinary Faculty at Complutense University of Madrid in Spain. She is the Director of the European Union Reference Laboratory for Bovine Tuberculosis and an expert on mammalian tuberculosis of the World Organization for Animal Health. Her research focuses on tuberculosis diagnosis, being involved in a national project on zoonotic tuberculosis.

Elisabeth Broens is an Associate Professor in Clinical Veterinary Microbiology at Utrecht University in the Netherlands. She is an EBVS Diplomate of the European College for Veterinary Microbiology, and her research focuses on zoonotic infections, antimicrobial resistance and antimicrobial stewardship in the context of One Health.

Jorge Pinto Ferreira is a Doctor of Veterinary Medicine with a Masters in Food Safety and a PhD in Population Medicine. His Doctoral studies were dedicated to antimicrobial resistance (AMR). In 2017 he also earned a diploma from the European College of Veterinary Public Health. Between 2012-2017, he worked as a consultant in Switzerland, (SAFOSO AG), and from October 2017 until June 2021 he worked for the World Organisation for Animal Health (OIE/WOAH), as the Deputy Head of the Antimicrobial Resistance (AMR) and Veterinary Products Department. He joined the Food and Agriculture Organization of the United Nations (FAO), in July 2021, as Food Safety Officer.

Maria Sofia Núncio, is a microbiologist, is a senior researcher at the National Institute of Health in Lisbon and Águas de Moura, Portugal. She is responsible for the Vector and Vector-Borne Disease Center and the Emergency Response and Biopreparedness Unit, and her research focuses on vector-borne diseases and emerging diseases caused by highly pathogenic microorganisms.

Zoonotic tuberculosis in Spain: surveillance and control through the One Health approach

Beatriz Romero[1], Javier Bezos[1], Julio Álvarez[1], Lucía de Juan[1], Lorente-Leal, V.[1], Laura Herrera[2], Zaida Herrador[2]

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Zoonotic tuberculosis (zTB) is a form of human tuberculosis acquired from an animal source. It is mainly caused by Mycobacterium bovis and M. caprae, and represents a global health problem linked to human-animal interaction. Although reported cases are scarce, recent studies estimate ~140,000 cases and 11,400 deaths annually worldwide, compared to only 371 cases reported to the WHO in 2023, reflecting significant underdiagnosis. Its incidence is higher in regions with raw milk consumption, limited veterinary control, and close contact with animals.

In Europe, zTB is relatively rare, but infection persists in domestic livestock and wildlife reservoirs, hindering eradication programs. The prevalence of positive cattle farms is generally low, but with persistent hotspots in certain countries and regions.

Spain is the EU country that has reported the most zTB cases in recent years, and it is being investigated whether this may be associated with herd-level prevalence of bovine tuberculosis. A national project carried out among public health and animal health institutions and authorities has shown that the causative agent of infection is identified in only 32.3% of all human tuberculosis cases. Of these, 4.3% are caused by M. bovis or M. caprae, mainly in elderly individuals, which may correspond to reactivations of past infections.

The detection of zTB reinforces the need to work under the One Health approach. Compliance with bovine tuberculosis eradication programs and the control of this infection in other reservoir species is essential. Furthermore, the data obtained highlight the underdiagnosis of zTB and the need for improvements in this field in the future.

Keywords

Zoonotic tuberculosis (zTB), bovine tuberculosis; underdiagnosis; One Health

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Emergence of *Brucella canis* in Europe: a threat to animal and public health?

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The zoonotic bacteria, *Brucella canis*, is becoming the leading cause of canine brucellosis in Europe. *B. canis* is mostly transmitted vertically from bitch to offspring or through vaginal discharge, semen or urine. In dogs, it causes reproductive problems as well as non-specific lameness or discospondylitis. The diagnosis of a *B. canis* infection in dogs is hampered by subclinical disease and nonspecific clinical signs. In addition, both serologic testing and bacterial isolation have their limitations because of the nature of the disease[1].

B. canis is considered endemic in the southern United States, Central America, and South America and has been reported from Canada, Asia, Africa, and Europe[2,3]. Recent papers have expressed concerns about the introduction of *B. canis* in countries to which it is not endemic through infected dogs from endemic areas. *B. canis* has been detected in dogs in almost all European countries which increased human exposure, but currently there is no systematic surveillance. Brucellosis in dogs is, in contrast to livestock, not notifiable to the World Organisation for Animal Health (WOAH). Moreover, *B. canis* caused brucellosis is not included in Animal Health Law, and therefore there is no legal framework to tackle this emerging infectious disease[4].

Humans can become infected with *B. canis* through direct contact with secreta and excreta of infected dogs or through laboratory exposure. Clinical signs in humans vary from subclinical infection to fever, malaise, splenomegaly, and lymphadenopathy. Host-*B. canis* interactions are still poorly understood, with many knowledge and capability gaps, causing relatively poor sensitivity and specificity of existing diagnostic tools. Therefore, the public health relevance of *B. canis* needs further investigation before a proper risk assessment can be performed [4,5].

Keywords

Brucella canis, public health, emergence, zoonosis

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Antimicrobial Resistance: Our One Health Challenge

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Antimicrobial Resistance (AMR) is one of the current top global health issues. Often, it is simplified to the version "resistance to antibiotics", and therefore associated by many only to human health. In reality, its dimensions are much broader, as all antibiotics are antimicrobials, but not all antimicrobials are antibiotics. Antimicrobials are used not only in human medicine but also in animals and plants/crops, with a spill over to the environment, making it a perfect illustration of a One Health issue.

In particular after the adoption of the Global Action Plan (GAP) on AMR in 2016, a multitude of initiatives have been implemented at different levels, many of them focusing on: i. raising awareness on AMR; ii. data collection and iii. responsible and prudent antimicrobial use, when needed. AT FAO, the AMR Action Plan is the overall umbrella, that covers, for example, the development of the InFARM (International AMR Monitoring System) database and the RENOFARM (Reduce the Need for Antimicrobials on Farms for Sustainable Agrifood Systems Transformation) initiative.

AMR can also be foodborne, and the same can be said the other way around: some of the foodborne infections are caused by antimicrobial resistant microorganisms. The exact dimensions of these phenomena are not quantifiable on a global scale. It is important to emphasize that AMR is also a food safety issue, that threatens future global food security. Some of the recent scientific findings challenge previously accepted knowledge regarding 'fitness cost" and the importance of controlling the development *vs* the transmission of AMR. The implementation of Codex AMR standards is nevertheless instrumental to ensure the protection of public health and fair trade.

The cornerstones to control AMR are: 1) a change in behaviors to preserve antimicrobials as global common goods and 2) an agrifood systems transformation that ensures sustainable food production.

Keywords

Antimicrobial Resistance (AMR); Antimicrobial Use (AMU); food safety; One Health

Interaction between climate change and vector-borne zoonoses

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Zoonotic diseases transmitted and shared between animals and humans have existed since the dawn of mankind[1]. Pathogens shared with animals are responsible for over 60% of infectious diseases in humans. Vector-borne diseases account for more than 17% of all infectious diseases, causing over 700,000 deaths annually. These diseases can be caused by parasites, bacteria, or viruses.

Climatic changes, landscape management, and the extensive transportation of humans, animals, and commodities significantly contribute to the spread of zoonoses. The silent dissemination and adaptation of vectors have further facilitated the expansion of vector-borne diseases[2].

Mosquitoes and sandflies are most common vectors in tropical areas, whereas in Europe, they act as zoonotic vectors only in a limited number in warmer areas[3]. Nonetheless, due to global warming and the consequent northward expansion of mosquito populations, diseases traditionally endemic to tropical zones have recently emerged in Europe. Currently, tick-borne zoonoses represent the most significant vector-borne diseases in Europe, with incidence rates increasing over the past decade.

In Portugal, Lyme borreliosis and Mediterranean spotted fever are the most prevalent zoonoses in humans. Recently, the detection of the first human case of Crimean-Congo hemorrhagic fever, a severe disease with mortality rates ranging from 30% to 60%, underscores the importance of surveillance and early detection of tick-borne diseases. Regarding mosquito-borne viruses, the only virus detected in humans and animals to date is West Nile virus, except for an outbreak of dengue on Madeira Island a few years ago[4]. Nevertheless, the occurrence of autochthonous cases of West Nile virus, dengue, and chikungunya in Europe highlights the necessity for continuous surveillance of mosquito-borne diseases.

Zoonoses pose an increasing public health challenge, as they are interconnected with environmental factors and human society. Complete eradication of vector-borne zoonoses remains a challenge. However, many vector-borne diseases are preventable through targeted protective measures and community engagement.

Keywords

Vector-borne zoonoses; climate change

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ORAL COMMUNICATION

Literacy in One Health – Urgency and Collaborative Challenges

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The interconnectedness of human, animal, and environmental health demands new approaches to literacy that move beyond sectoral silos[1]. Limited awareness of zoonotic and foodborne diseases, professional hazards and risk behaviors often hinders the effectiveness of prevention strategies. This presentation proposes a One Health literacy framework, conceptualized to strengthen the capacity of communities and professionals to recognize and mitigate shared health risks.

As a practical case, Hepatitis E virus (HEV) is used to illustrate the urgency and complexity of One Health literacy. HEV (genotype3) is transmitted through foodborne routes (consumption of undercooked traditional staples, such as pork products, vegetables and mollusks), occupational exposure (e.g.: farmers, veterinarians, slaughterhouse workers, forestry operatives, field biologists), and environmental pathways. These multiple risk dimensions highlight the necessity of targeted literacy strategies tailored to distinct groups: (i) professional sectors at risk, (ii) consumers with traditional dietary habits, and (iii) youth educators.

The proposed literacy framework involves a multidisciplinary team—including veterinarians, physicians, biologists, food safety experts, social scientists, communication specialists, and educators—working collaboratively to:

- Map risk groups and literacy gaps through participatory needs assessments.
- Design tailored interventions, such as visual tools, culturally adapted communication materials, training workshops, and digital resources.
- Implement pilot literacy actions in professional and community settings.
- Evaluate effectiveness through mixed methods: pre- and post-intervention knowledge assessments, behavioral indicators (e.g., hygiene and food preparation practices), and participatory feedback from target groups.

This framework underlines literacy as a cornerstone of One Health capacity building, fostering informed decision-making and risk reduction at individual, community, and professional levels, whilst promoting sustainable animal health and production systems, and healthy natural environments[2, 3]. By using Hepatitis E as an example, the presentation aims to advance the dialogue on urgency, challenges, and collaborative solutions for implementing literacy strategies that bridge human, animal, and environmental health.

Keywords

Health Literacy, Multidisciplinarity, Capacity-Building, Governance

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Backyard production systems in Portugal: Exploring Associated Risk Factors and Antimicrobial Resistance through a One Health lens

Ana Esteves[1,2], Carlos Aniceto[3], Bruno Baroni[1], Sónia Ramos[2,4], Rita Ferreira[1], Daniel Sobral[1], João Dourado Santos[1], João Paulo Gomes[1,2,4], Ausenda Machado[3], Alexandra Nunes[1,2,4]

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Antimicrobial-resistant (AMR) zoonotic bacteria are a growing global health threat, potentially transmitted via food, animal contact, and the environment. Backyard production systems (BPS) are particularly concerning due to close human-animal contact, limited veterinary oversight, poor waste management, unregulated antibiotic use, and low awareness of zoonotic risks. Untreated manure and interactions with wild and companion animals can further facilitate the spread of antimicrobial resistance genes (ARGs). In Portugal, despite BPS legal registration requirements, compliance is low, hindering risk assessment and surveillance. This study aimed to evaluate risk factors linked to BPS management practices and the presence of AMR markers.

A nationwide epidemiological questionnaire collected information on BPS owners' demographics and husbandry practices to identify associated risk factors. Samples were also collected from 10 selected farms across different NUTS III regions, including poultry, small ruminants, pigs, and soil from animal-accessible areas and vegetable gardens. A probe-based metagenomics approach was used to identify AMR markers circulating within and between farm agroecosystems.

Almost 600 responses were collected through the questionnaire. The findings indicate that most BPS owners consume eggs and poultry meat. Notably, 19% reported administering antibiotics to their animals without a veterinary prescription, 51% vaccinated their animals, and 81% sought advice from veterinarians.

Metagenomic analysis revealed the presence of multiple AMR markers across the collected samples. Differences in AMR marker diversity were observed both within and between animal and soil sample groups.

This study is the first of its kind conducted in Portugal, providing a comprehensive characterization of BPS and insights into local husbandry practices. The detection of antimicrobial resistance (AMR) markers in these settings lays the groundwork for investigating the transmission dynamics of ARGs within BPS.

Keywords

Backyard Production Systems, Public Health, One Health, Metagenomics

Trichinella Detection in Wild Boar Meat Private Consumption - Insights from a European Study

Mariana Rodrigues[1,2,3], Vincenzo Veneziano[4], Famke Jansen[5], Thomai Lazou[6], Sorin Daniel Dan[7], Miroslaw Rozycki[8], Jelena Petrovic[9], Madalena Vieira-Pinto[1,3,10]

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The private consumption of wild boar meat is increasingly widespread across Europe, raising public health concerns due to the risk of Trichinella spp. infection. This study presents the results of a cross-sectional survey conducted in 26 European countries, assessing legislation, practices, prevalence data, and awareness measures regarding Trichinella detection in wild boar meat intended for private consumption. Findings reveal significant heterogeneity in testing obligations, sample handling procedures, laboratory requirements, and enforcement mechanisms. While some countries mandate systematic testing and subsidize costs, others rely on voluntary testing or impose no requirements, leaving consumers at higher risk. Reported prevalence of Trichinella in wild boar varied widely, with values ranging from

<0.001% to over 6% depending on the country and year, though data availability was often inconsistent. Awareness campaigns and hunter training remain limited in many regions, contributing to underreporting, low compliance, and insufficient risk perception. Key challenges identified include a lack of hunter engagement, insufficient testing facilities, and variable penalties for non-compliance. To mitigate risks, the study recommends harmonizing surveillance strategies, expanding education and communication initiatives, ensuring accessible and affordable testing, and strengthening collaboration between veterinary authorities and hunting communities. Such measures are essential to reduce the zoonotic risk of trichinellosis linked to privately consumed wild boar meat in Europe.

Keywords

Private Consumption, Trichinella detection, Wild boar, Zoonosis

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Tracing the invisible threat: spatial epidemiology of *Brucella suis* in Northeast Portugal

Zita Martins Ruano[1,2,3], Teresa Letra Mateus[1,2,3,4, *], José Aranha[5,6], Eirini Fragkiadaki[7], Georgia Tzouganatou[8], Emmanouil Angelakis[8], Luís Félix[5,6], Jorge Pereira[9], Ana Chorense[10], Claudia Rocha[10], Dulce Loureiro[10], Ricardo Marcos[10], Madalena Vieira-Pinto[1,2,3,11,12]

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The interface between wild boar and domestic pigs is a critical hotspot for the bidirectional transmission (spillover and spillback) of various diseases, notably brucellosis. This cross-sectional study investigated the seroprevalence of *Brucella* spp. and *Brucella suis* among swine populations at the wildlife-livestock interface in northeast Portugal, focusing on wild boar and the autochthonous Bísaro pig breed. Antibodies against *Brucella* spp. were found in 25.7% of wild boar (95/370; 95% CI: 21.2%–30.1%) and 15.2% of Bísaro pigs (35/231; 95% CI: 10.5%–19.8%). Of these, 82 wild boar and 33 domestic pigs tested positive for *B. suis*, with molecular confirmation in three domestic pigs—marking the first molecular identification of *B. suis* in Bísaro pigs. Spatial analysis revealed distinct prevalence patterns: higher rates in wild boar in the northwest and in domestic pigs in the southeast. A significant positive correlation between the spatial distributions of wild and domestic swine suggests potential transmission at the interface. Environmental and anthropogenic factors showed moderate correlation with *B. suis* prevalence, indicating a possible link to animal overpopulation. Additionally, prevalence was positively associated with farm land elevation and negatively with dense shrubland coverage, underscoring the influence of farming practices on pathogen spillover. These findings emphasize the multifactorial nature of *B. suis* distribution, shaped by ecological and human-driven landscape features. Given its zoonotic potential, this study highlights the urgent need for integrated One Health surveillance and control strategies to mitigate public health risks in rural communities and protect both animal and human health at regional and national levels.

Keywords

Brucellosis, wild boar, Bísaro pig, zoonotic spillover

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Anisakiasis: The Importance of Stakeholder Engagement in the Zoonotic Disease Control

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The black scabbardfish, *A. carbo* is frequently found heavily parasitized with *Anisakis* nematode larvae. The Innovation Pact, a project known as the "Blue Bioeconomy Pact," aims to study the parasitic infection of this fish and, based on the results obtained, outline recommendations to support the industry throughout the black scabbardfish value chain. Four batches of black scabbardfish (n=120) were obtained seasonally, and the prevalence of *Anisakis* nematodes was 100%. Our data showed no significant seasonal variation in the number of worms, and a higher load of larvae was observed in the visceral cavity and internal organs. Although the parasite number in the dorsal muscle is low, the unpredictability of the presence of the zoonotic agents *A. simplex* s.s. and *A. pegreffii* poses a potential health risks with consumption raw or undercooked black scabbardfish. Based on the epidemiological data obtained, a set of control measures and increased consumer awareness was recommended for improvement and/or implementation throughout the black scabbardfish value chain, aiming to increase the safety and quality of the fishery product and the sustainability of its capture. The outputs of each measure were predicted and presented.

From the "One Health Integration" perspective, the authors reinforced the importance of stakeholder engagement to improve disease reporting systems and raise questions about the management of this information.

Keywords

Anisakiasis, Food Safety, Black scabbardfish, Value Chain Recommendations

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Insights into management and outcomes of hospitalization due to infected animal bites: a retrospective study at a tertiary hospital in Portugal

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Background: Animal bites, although generally uncomplicated, may warrant hospitalization, particularly due to the need for surgical correction and/or intravenous antibiotic therapy for infection.

Methods: Retrospective observational study including all patients with at least one hospitalization at the São João Hospital due to a non-human vertebrate bite with signs of skin and soft tissue infection between 01/2016 and 12/2022. Research conducted using ICD-9 and ICD-10 discharge diagnosis codes. Systematic analysis of clinical records was performed.

Results: Fifty-six patients were identified, with a median age of 54 years (11% were children); 41% were female. Twenty percent of patients had immunosuppression factors, mostly diabetes mellitus. The median length of hospital stay was 9 days (range 1-54). Bites by cats (48%) and dogs (41%) were the most common. In most cases, the bite was single (84%), most commonly in the upper limbs (67%) (especially hands - 45%). Systemic antibiotic prophylaxis was performed in 46% of patients. As a complication of skin infection, 25% of patients had abscess/collection, 21% tenosynovitis, 13% arthritis, 11% osteomyelitis, and 4% bacteremia. Tenosynovitis was more frequent in cat bite infections and osteoarticular involvement in dog bites, but these differences were not statistically significant (p=0.158 and p=0.321, respectively). Forty-six percent of patients underwent surgical intervention (more frequently in dog bites, p=0.012). All patients received systemic antibiotic treatment, initiated at a median of 5 days after the bite. The median duration of antibiotic therapy was 16 days (range 5-232). Amoxicillin/clavulanic acid was the first-choice antibiotic. Microbiological samples were collected in 75% of patients, with an agent identified in 31% of these. Four percent of patients died (n=2).

Conclusions: Use of appropriate antibiotic prophylaxis after animal bites was still frequently insufficient. Raising awareness of the risk of bite infection among pet tutors and emergency professionals, under a One Health lens, could help overcome this situation. Deep-seated complications were common, requiring adequate clinical evaluation and imaging for identification and management; nearly half of the patients needed surgical intervention, highlighting the importance of prompt and comprehensive care.

Keywords

Animal bite; wound infection; bacterial infection; One Health

Beyond Hospitals: The Role of Companion Animals and Veterinary Healthcare Facilities in the Epidemiology of Community-Acquired *Clostridioides difficile*

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Clostridioides difficile is a spore-forming anaerobe that colonizes the intestinal tract of various animal species and persists in environmental reservoirs. The increasing incidence of community-acquired *C.difficile* infection (CA-CDI) reflects an epidemiological shift from healthcare-associated to community-based transmission. This study aims to characterize *C. difficile* strains circulating within canine and feline populations, and to assess environmental contamination in veterinary healthcare facilities.

To ensure representativeness, sampling is being conducted in three veterinary hospitals located in different Portuguese regions. Rectal swabs are randomly obtained from animals attending the hospitals, while sponge swabs are used to sample near-patient surfaces. All samples undergo broth enrichment for one week and are cultured on selective media. Suggestive colonies are confirmed by MALDI-TOF. Toxigenic profiling and genetic diversity are assessed via multiplex PCR and PCR ribotyping. Antimicrobial susceptibility testing is performed on all isolates.

From May 2025 to the present, a total of 59 animal and 46 environmental samples had been collected from a single veterinary hospital. The overall prevalence in animals was 27.3% (14/59), with rates of 26.6% in cats and 22.7% in dogs. Environmental contamination was detected in 8.7% (4/46) of samples. Of the positive animals, 71.4% (10/14) were colonized with toxigenic strains, while all environmental isolates were toxigenic. The most prevalent toxigenic ribotypes found were RT106 (23.5%) and RT014/020 (17.6%), both commonly reported in humans in Portugal. Significant resistance rates were observed to clindamycin (35.3%) and moxifloxacin (23.5%).

The detection of toxigenic *C. difficile* in companion animals and in the veterinary hospital environment highlights the potential role of animals in the epidemiology of CA-CDI. These findings also suggest the presence of antimicrobial resistance in *C. difficile* strains from companion animals. Overall, our data suggests that dogs and cats may serve as reservoirs for human-associated strains, and that shared environments might contribute to the transmission cycle.

Keywords

Clostridioides difficile, companion animals, veterinary hospital, One Health

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DNA Aptamers Targeting F4-Fimbriae to Block Adhesion of Enterotoxigenic *Escherichia coli*

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Enterotoxigenic *E. coli* (ETEC) infections lead to significant costs for the swine industry worldwide[1]. Among other virulence factors, fimbriae are essential for the initial adhesion of ETEC to intestinal epithelial cells. In particular, F4- type (K88) fimbriae are commonly associated with neonatal infections and most post-weaning diarrheal infections[2]. These infections are traditionally prevented or treated with antibiotics, but their use is highly restricted due to antimicrobial resistance[3,4]. Therefore, DNA aptamers emerge as a promising approach to block the initial adhesion of F4-ETEC due to their target-binding capacities[5].

The present study was divided into four main steps: (1) selection of specific aptamers against F4-ETEC bacteria using the cell-SELEX approach; (2) evaluation of dissociation constant (K_D) for the selected aptamers (AptF4_1, AptF4_2, AptF4_3, AptF4_4) (3) evaluation of *in vitro* specificity for these aptamers using two different bacterial strains: F4- ETEC and *E. coli* K12 (commensal *E. coli*); (4) evaluation of the anti-ETEC performance of the most specific aptamers (AptF4_1, AptF4_2) using the *Galleria mellonella* model.

From the cell-SELEX methodology, the four most representative aptamers were selected from a total of 2.5 million sequences sequenced by NGS. These aptamers presented low K_D values, indicative of high binding affinities with F4- type fimbriae. Regarding the *in vitro* specificity evaluation, one aptamer (AptF4_2) stood out due to its higher specificity to F4-ETEC in comparison with *E. coli* K12. The *in vivo* tests corroborated the anti-ETEC potential of AptF4_2 because this aptamer at 500 nM increased the survival and health index of *Galleria mellonella* compared to the positive control (F4-ETEC inoculation).

Therefore, this work demonstrates the potential of aptamers in the treatment of ETEC infections as an alternative to antibiotics. However, for future swine applications, these aptamers will be modified with nucleic acid mimics and encapsulated for oral administration.

Keywords

ETEC, Aptamers, Galleria mellonella

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Antimicrobial Potential of *Opuntia ficus-indica* Phenolics Against Bovine Mastitis Pathogens and Their Transfer to Milk: Implications for One Health

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The rising prevalence of antimicrobial resistance (AMR) among mastitis-related pathogens represents a major challenge for animal health, food safety and public health, reinforcing the need for integrated One Health solutions to control zoonotic risks. *Opuntia ficus-indica* (OFI), a cactus crop traditionally used as forage in arid regions, contains phenolic compounds (PhC) with demonstrated antibacterial activity. Using these bioactive compounds in dairy production could reduce antibiotic inputs, mitigate AMR emergence and lower the potential transmission of mastitis-associated zoonotic bacteria through milk and dairy products. This study aimed to evaluate the antibacterial activity of OFI cladodes against mastitis-related pathogens, isolate the PhC, and determine whether these activities are transferred into milk in OFI-fed cattle. Fresh OFI cladodes (FCla) were milled to a pulp, and PhC-peaks were separated through RP-HPLC after ethanolic extraction. Antibacterial activities were assessed using a microdilution assay against clinical and commensal bacterial strains, including *Pseudomonas spp.*, *Staphylococcus spp.*, *Streptococcus spp.*, *Escherichia coli*, and *Klebsiella spp.* (n=12). Milk samples were collected from two livestock groups (n=2): (a) a test group fed with FCla-enriched feed, and (b) a control group fed with standard feed, matched for all other conditions. Phenolic compounds in milk were identified and antibacterial activity in milk was re-evaluated using the same bacterial strains and methods. FCla- pulp and PhC-peaks presented significant and dose-dependent antibacterial activities ($p<0.001$). PhC profiles in milk from FCla-fed livestock showed OFI phenolic peaks absent from control milk. Additionally, milk from FCla-fed livestock presented identical bioactivities to those determined for FCla-pulp, indicating that the bioactive antibacterial PhC was transferred to milk unaltered by digestion. These findings position OFI as a promising natural alternative to antibiotics in dairy systems, contributing to the control of mastitis and associated zoonoses, and supporting AMR mitigation within the One Health framework.

Keywords

Opuntia ficus-indica, Mastitis, Antimicrobial resistance, Zoonoses, One Health

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Emergence of *Gasterophilus pecorum* in the Equine Duodenum: Climate Sentinel and One Health Implications

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Gasterophilus spp. are obligate parasites of equids, with nine recognized species exhibiting distinct geographical distributions. Their larval stages inhabit the gastrointestinal (GI) tract of horses, inducing variable degrees of mucosal inflammation.[1] Among them, *Gasterophilus pecorum* is considered one of the most pathogenic species, yet its prevalence in Portugal remains low. The present report describes a rare case of duodenal myiasis caused by *G. pecorum* in a 6-year-old Lusitano mare, presented in April 2024 to the Equine Veterinary Teaching Hospital (HVE-FMV-Univ. Lusófona, Lisbon) for gastroduodenoscopy due to poor performance, girth sensitivity, and right-sided flank pain.

Endoscopic evaluation revealed Grade 1/4 lesions (Consensus Statemen 2015) in the squamous gastric region, with mild to moderate pyloric mucosal erythema and erosions. In the duodenum, moderate to severe diffuse enanthematous and diphtheric lesions, with focal erosions and nodular hyperplasia, were observed. Larvae of *G. pecorum* were identified adherent to these lesions. Histopathology confirmed lymphoplasmacytic and eosinophilic enteritis with mucous metaplasia and epithelial hyperplasia. Coprological analysis showed 400 eggs per gram (EPG) nematodes. Endoscopic removal of larvae was performed, followed by oral administration of ivermectin paste (200 µg/kg b.w.). Clinical improvement was documented.

In Europe, G. intestinalis is the most prevalent species, while *G. haemorrhoidalis* and *G. nasalis* are also widespread.[2,3] The presence of *G. pecorum* in the duodenum of a horse from a low-prevalence region such as Portugal raises epidemiological concern. Previous surveys reported <3% prevalence in restricted areas of Eastern and Central Europe.[4] The parasite's development is influenced by climatic conditions, displaying seasonal dynamics.[5] Its emergence in atypical anatomical sites and geographical contexts may reflect adaptive capacities and shifting ecological niches.

This case highlights the importance of vigilant parasitological surveillance, integrating equine welfare, environmental monitoring, and zoonotic risk under the One Health approach.

Keywords

Gasterophilus pecorum, equine, duodenum, One Health

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Crimean-Congo Hemorrhagic Fever in Portugal: Epidemiological and Clinical Insights from a Fatal Case, Bragança 2024

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Crimean-Congo haemorrhagic fever (CCHF) is a severe tick-borne disease caused by *Orthonairovirus haemorrhagiae*, with wide distribution across Africa, Asia, the Middle East, and Europe[1]. The first European case was reported in Bulgaria in the 1950s, followed by emergence in several Balkan and Mediterranean countries. Autochthonous cases have since been reported in Greece (2008)[2] and Spain (2016)[3], confirming the virus's geographic expansion. In Portugal, serological evidence of exposure was detected in 1985[4], but the virus had never been identified.

On 12 July 2024, an 83-year-old man from Bragança, without travel history, developed fever and myalgia following a tick bite. Initially discharged from the local hospital with suspected Mediterranean spotted fever (MSF) and prescribed doxycycline, his condition deteriorated, and he was readmitted with progressive symptoms. Despite treatment, he developed multiorgan failure and died on 22 July. As the cause of death was unclear, a serum sample was retrospectively tested at the national reference laboratory, confirming CCHF infection through viral RNA detection and specific IgM antibodies[5]. Genetic analysis identified genotype III/Africa 3, closely related to strains circulating in Spain and several African countries.

This case confirms the circulation and public health risk of CCHFV in Portugal, prompting an immediate alert and underlining the need to consider CCHF in the differential diagnosis, namely in suspected MSF cases unresponsive to specific therapy. While CCHF is often associated with severe disease and high mortality, milder infections may go undetected. Given its high biosafety risk, handling and diagnosis require BSL-4 conditions, alongside strict patient containment. Rapid diagnostic capacity is essential, and preventive measures to reduce tick exposure (repellents, protective clothing, and awareness campaigns) should be reinforced, particularly among high-risk groups such as farmers, hunters, and outdoor workers. These findings highlight the need for vigilance and strengthened surveillance in at-risk regions.

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Keywords

Crimean-Congo Hemorrhagic Fever, Viral Hemorrhagic Fevers, Vector Borne Diseases, Portugal

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Ticks (Ixodidae) collected on bovines and tick-borne pathogens at traditional housing systems in Huambo, Angola

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Ticks (Acari: *Ixodidae*), one of the main blood-feeding ectoparasites of livestock, are competent vectors of a wide variety of pathogens of great economic importance in production, but they are also of great importance in terms of human health due to the zoonotic potential of some of these pathogens. In tropical regions, agricultural communities face an increased risk of exposure, since the daily management of production systems is carried out by children or the elderly, raising additional concerns. 345 ticks were collected from 25 bovines (4 males and 21 females) belonging to different small farmers in Huambo, Angola. The tick specimens were identified morphologically, with 137 being processed for genomic DNA extraction and screened for the presence of tick-borne pathogens, namely *Babesia* and *Theileria* (target: 18S rRNA gene), *Anaplasma* and *Ehrlichia* (target: 16S rRNA gene), as well as *Rickettsia* (targets: 16S rRNA and gltA). 97% of the tick specimens collected belong to the genus *Rhipicephalus*, with 75.4% belonging to the subgenus *Boophilus* and 22.3% to the subgenus *Rhipicephalus*. A small percentage was identified as *Amblyomma* (1.4%) or *Hyalomma* (0.9%). In screening for the presence of pathogens, we have so far identified the following pathogens in *R. Boophilus* samples: *Babesia bigemina* (4), *Babesia microti* (2), *Theileria mutans* (5), *Theileria velifera* (1), *Anaplasma marginale* (3), *Anaplasma capra* (2), *Anaplasma platys* (3) and *Rickettsia africae* (4). *B. microti*, *A. capra* and *R. africae* have zoonotic potential and pose a significant risk to the farming communities.I the increasing urbanisation and the proximity of human populations to large farms create ideal conditions for the transmission of pathogens between animals and humans. In this context, it is essential to promote coordinated action between medical, veterinary, ecological and public health professionals to create effective surveillance, prevention and control strategies.

Keywords

Ticks, bovines, Angola

POSTER

REVIVE: 10 Anos de Monitorização pela Rede Vigilância de Vetores no Alto Tâmega e Barroso

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Entomological surveillance is essential for the prevention of vector-borne diseases, especially in high-risk regions such as Alto Tâmega e Barroso. This paper presents the results of 10 years (2015-2024) of systematic monitoring of ixodids (ticks) and mosquitoes (adults and immatures) in the six municipalities of the region, as part of the REVIVE programme - National Vector Surveillance Network.

871 records of ixodids were analysed, 529 of adult mosquitoes and 739 of immature mosquitoes, collected in Boticas, Chaves, Montalegre, Ribeira de Pena, Valpaços and Vila Pouca de Aguiar. The collection was carried out in multiple habitats, with georeferencing and environmental characterisation, making it possible to identify relevant spatial and temporal patterns.

Ixodids were most abundant in 2023 (115 records) and 2021 (101 records), with dogs predominating as the main host, followed by humans and other domestic animals. For adult mosquitoes, the years 2023 and 2024 stood out for having the highest number of records (76 and 70, respectively), while for immature mosquitoes the peaks occurred in 2023 (116 records) and 2024 (85 records).

Analysing the data shows a heterogeneous distribution of vectors between municipalities and over time, with variations associated with environmental factors, farming activities and the possible effects of climate change. Continuous monitoring has made it possible to identify hotspots of abundance and critical periods of activity. Inter-institutional collaboration is essential for this continuous monitoring.

These results reinforce the relevance of Alto Tâmega e Barroso as a strategic region for vector surveillance in Portugal, contributing to the anticipation of risks and the implementation of preventive measures integrated into the ‘One Health’ approach.

Keywords

Entomological surveillance, Vectors (ixodid ticks and mosquitoes), Public health, Climate change

Wild Boar Surveillance for *Francisella tularensis* in Central Portugal: Low Seroprevalence, High Relevance

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Tularemia is a zoonotic disease caused by the highly contagious bacterium *Francisella tularensis*, which has been reported in humans in Portugal. It affects more than 250 animal species, with rabbits, hares, and rodents being the primary reservoirs. Clinical signs in humans can range from mild to life-threatening, depending on the route of exposure (ingestion, inhalation, skin contact, injection, or tick bite), with high fever being a common feature. In a One Health framework, understanding the role of wildlife in the epidemiology of zoonoses is essential for early detection and prevention strategies. To investigate the potential involvement of wild boar (*Sus scrofa*) in the transmission cycle of *F. tularensis* in Portugal, a surveillance study was conducted using serum and organ samples from 368 wild boar collected in the central region of the country. A commercial enzyme-linked immunosorbent assay (ELISA; IDVet®, Montpellier, France) was used to detect specific antibodies against *F. tularensis*, and a conventional PCR targeting the 16S rRNA gene was applied to organ samples for molecular detection. Antibodies to *F. tularensis* were detected in one adult male wild boar, corresponding to a seroprevalence of 0.27% (95% confidence interval [CI]: 0.003–1.5%). All organ samples tested negative by PCR. These findings suggest that wild boar are exposed to *F. tularensis* in central Portugal, although active infection was not detected in tissues. Despite the low seroprevalence observed, the zoonotic potential of this pathogen warrants continued surveillance. The integration of wildlife monitoring into public health strategies is crucial to anticipating and mitigating the risk of transmission to humans and domestic animals, in line with One Health principles.

Keywords

Francisella tularensis; Occurrence; Wild boar

Acknowledgements

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Fermentation-Derived Peptides as Antibiotic Alternatives for Sustainable Animal Health: Targeting Foodborne Zoonotic Pathogens within a One Health Framework

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Reducing antibiotic use in livestock is a global priority to mitigate antimicrobial resistance and protect human, animal, and environmental health. Foodborne zoonotic pathogens pose significant risks to public health and food safety, particularly in intensive production systems. Bioactive peptides generated through microbial fermentation are emerging as sustainable alternatives to antibiotics, with the potential to inhibit zoonotic pathogens and support gut health, aligning with the One Health concept.

This study aimed to characterize a novel fermentative hydrolysis using a *Bacillaceae* species (identity withheld for confidentiality) that produces peptides with antibacterial activity against key foodborne pathogens and the potential to modulate the gut microbiome. Fermentation was performed in milk for 48 hours. Coagulated proteins were removed by filtration, and peptides were isolated from the soluble fraction by ultrafiltration. Antibacterial activity was assessed against *S.aureus*, *L.monocytogenes*, *S.enterica*, *E.coli* O157 and *B.cereus* using broth microdilution assays, and minimum inhibitory concentrations (MICs) were calculated. Gut microbiota modulation was evaluated in vivo in mice using 16S rRNA gene sequencing.

The peptides exhibited dose-dependent inhibition of all tested pathogens, with MICs ranging from 3–6 µg/mL. In vivo, peptide administration induced beneficial shifts in gut microbial composition, enhancing the abundance of health-associated taxa and overall microbial diversity. These results indicate that fermentation-derived peptides are resistant to digestive degradation and demonstrate a dual bioactivity profile: limiting pathogenic bacterial growth while promoting gut microbial resilience, critical for sustainable livestock production and mitigation of foodborne zoonoses. These findings support the development of fermentation-derived peptides as natural antibiotic alternatives, contributing to animal health, food safety, and public health, consistent with One Health principles.

Keywords

Fermentation-derived peptides, Antibiotic alternatives, Livestock health, One Health

Acknowledgements

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Characterization of *Staphylococcus aureus* in Raw Meat: Antimicrobial Resistance and Virulence Gene Profile

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Staphylococcus aureus is a major public health concern due to its ability to cause infections, produce virulence factors, and develop antimicrobial resistance. This study aimed to assess the occurrence of *S. aureus*, its virulence genes, and antimicrobial resistance profiles in raw meat from local establishments and supermarkets. A total of 34 raw meat samples (9 meat rolls and 25 hamburgers/meatballs) were collected. Coagulase-positive *Staphylococcus* (CoPS) were enumerated on Baird-Parker agar according to ISO 6888-1:2021. DNA was extracted from seven representative isolates (3 meat rolls, 4 hamburgers/meatballs), and PCR was used to detect the species-specific *nuc* gene, virulence genes (*hla*, *tst*), and enterotoxin genes (*sed*, *seg*, *sei*). Antimicrobial susceptibility was assessed using the Kirby-Bauer disk-diffusion method against a panel of 14 antibiotics (penicillin, oxacillin, cefoxitin, gentamicin, tetracycline, erythromycin, clindamycin, rifampicin, imipenem, linezolid, mupirocin, chloramphenicol, ciprofloxacin, and sulfamethoxazole/trimethoprim), following the EUCAST guidelines. Average CoPs counts were 0.11 ± 0.15 $\log_{10}$ CFU/g in meat rolls, and 0.57 ± 0.09 $\log_{10}$ CFU/g in hamburgers/meatballs, below the regulatory limit of 10^2 CFU/g. The *nuc* gene was detected in all isolates, and all isolates tested carried the *hla*, *sei*, *seg*, and *tst* genes. Antimicrobial resistance was most frequent for gentamicin (3/7; 42.9%), tetracycline (3/7; 42.9%), oxacillin (2/7; 28.6%), penicillin G (2/7; 28.6%), erythromycin (2/7; 28.6%), clindamycin (2/7; 28.6%), and rifampicin (1/7; 14.3%). All isolates were susceptible to sulfamethoxazole/trimethoprim, linezolid, mupirocin, chloramphenicol, and ciprofloxacin. Methicillin-resistant *S. aureus* (MRSA) was identified in two isolates (beef and chicken burger; 28.6%), which also showed multidrug resistance (MDR), resistance to ≥ 3 classes of antibiotic. MRSA and MDR *S. aureus* strains are present in raw meat even when bacterial counts are below regulatory limits. Continuous surveillance and strict hygiene practices are essential to reduce foodborne transmission and mitigate the public health threat of antimicrobial resistance.

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Cardoon (*Cynara cardunculus*) Rennet as a Natural Intervention Against Foodborne Zoonotic Bacteria in Traditional Cheese

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Cardoon thistle (*Cynara cardunculus*), commonly known as Cardo in Portugal, is used in a water-based extract, thistle-rennet, as a milk coagulant alternative to animal rennet in traditional cheese production. This practice is particularly relevant for cheese industries in Serpa (Alentejo), Serra da Estrela, and Azeitão. Despite the well-characterized pharmacological properties of other thistle species, the antimicrobial potential of cardoon or thistle-rennet remains largely unexplored. Evaluating these properties is critical for enhancing food safety, mitigating foodborne zoonotic risks, and supporting functional food development. This study aimed to investigate the antibacterial activity of thistle-rennet against major foodborne pathogens relevant to cheese production. Cardoon was collected from a cheese facility in Serpa, and thistle-rennet was prepared in aqueous solution according to standardized procedures. Antibacterial activity was assessed against *Escherichia coli*, *Listeria spp.*, *Salmonella spp.*, and *Staphylococcus aureus* using standard microdilution assays. Thistle-rennet exhibited dose-dependent inhibition of all tested bacteria, with growth reductions ranging from 40–75% for *E. coli*, 45–70% for *Listeria spp.*, 50–72% for *Salmonella spp.*, and 42–68% for *Staphylococcus aureus* at the highest tested concentrations, all statistically significant compared to controls ($p<0.01$). These results indicate that cardoon-derived rennet may effectively limit the survival of foodborne pathogens in cheese, enhancing product safety and mitigating zoonotic risks. Given its widespread use in the food industry and GRAS status, cardoon represents a promising natural intervention aligned with One Health principles, integrating animal production, food safety, and human health.

Keywords

Cynara cardunculus, Cardoon, Cheese production, Foodborne pathogens, Zoonoses, One Health

Acknowledgements

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Domestic Reptiles as Asymptomatic Reservoirs of zoonotic bacteria: Risks to Public Health (Snakes)

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Exotic animals are increasingly recognized as reservoirs of zoonotic pathogens, yet their role in transmission remains underestimated. Reptiles, particularly snakes, have become popular companion animals, often without owners realizing they may carry bacteria such as Salmonella spp. and Escherichia coli, frequently linked to antimicrobial resistance (AMR) genes. The lack of legislation and hygiene measures heightens the public health risk, reinforcing the need for a One Health perspective. This study aims to detect and characterize zoonotic bacteria in domestic snakes, focusing on potential transmission pathways and associated AMR determinants.

A total of 154 oral and cloacal swabs were collected from 77 domestic snakes in Portugal. Samples underwent selective enrichment and culture, and bacterial identification was performed using MALDI-TOF MS. Salmonella-positive isolates were further analyzed by whole genome sequencing (WGS) for genomic characterization.

From 77 domestic snakes, 380 Gram-negative bacteria were isolated, 75.5% (n=287/380) from cloacal samples and 24.5% (n=93/380) from the oral cavity. Overall, 25 bacterial species were identified, with E. coli (40.5%; n=154), Salmonella enterica (17.1%; n=65) and Klebsiella pneumoniae (10.3%; n=39) most frequent. S. enterica was detected in 49 snakes (61 cloaca, 4 oral cavity). Co-infection was common, as 83.7% (n=41) of positive snakes

also harbored other species. In silico analysis identified 19 distinct serotypes, with S. enterica subsp. diarizonae ser. 47:z10:z35 (ST4578) most common, followed by S. enterica subsp. enterica Paratyphi B (ST88) and S. enterica subsp. salamae Iib 48:i:z (ST574). Importantly, multidrug-resistant (MDR) bacteria such as ESBL/AmpC- or carbapenemase-producing Salmonella spp. were not detected. Most isolates were susceptible to all antimicrobials tested.

Domestic snakes can carry zoonotic bacteria, including Salmonella enterica serovar Paratyphi B, highlighting their potential risk to public health and the need for One Health–based preventive measures.

Keywords

Zoonoses, One Health, Zoonotic bacteria, Exotic animals

Foodborne Pathogens and Antimicrobial Resistance in Poultry Meat: Insights from Lisbon Retail

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Salmonella spp., *Campylobacter spp.*, and *Escherichia coli* are common foodborne pathogens in poultry meat, posing significant public health risks, underscoring the importance of continuous investigation in the context of food safety and antimicrobial resistance (AMR).

A total of 39 poultry meat samples were purchased from supermarkets in the Lisbon area. Each sample (25 g) was analyzed for the presence of *Salmonella spp*. (IRIS *Salmonella*® method validated by AFNOR) and *E. coli*. For *E. coli*, enrichment medium was used before adding the selective supplement. Eighteen samples were also tested for *Campylobacter spp*., following ISO 10272-1:2017. Suspected isolates were identified by MALDI-TOF. *C. jejuni* and *C. coli* isolates were tested for susceptibility to seven antibiotics using the disk diffusion method, according to EUCAST and Société Française de Microbiologie criteria.

Salmonella spp. was detected in 7.7% (3/39) of the samples, and *E. coli* in 79.5% (31/39). *Campylobacter* spp. was isolated from 83.3% (15/18), with 60% of positive samples yielding multiple strains. In total, 14 *C. jejuni*, 11 *C. coli*, and 1 *C. lari* were identified. Regarding AMR, only five isolates (3 *C. coli* and 2 *C. jejuni*) were susceptible to all tested antibiotics. Approximately 44% (6 *C. coli* and 5 *C. jejuni*) exhibited multidrug resistance (MDR) to ciprofloxacin, tetracycline, erythromycin, gentamicin, and ampicillin. None of the MDR isolates showed reduced susceptibility to amoxicillin-clavulanic acid. Other detected bacteria included *Klebsiella pneumoniae* (7.7%; 3/39), *Pseudomonas aeruginosa* (10.3%; 4/39), *Aeromonas veronii* (23.1%; 9/39), and *Citrobacter* spp. (7.7%; 3/39).

These findings highlight the importance of preventive measures in poultry farms, slaughterhouses, and retail, including hygiene practices, vaccination, HACCP implementation, and traceability systems. Such strategies are crucial to mitigate contamination and AMR in poultry meat, contributing to improved food safety and public health within a One Health framework.

Keywords

Poultry meat, Foodborne pathogens, Antimicrobial resistance, Food safety

Awareness of livestock veterinarians on livestock Q fever – First evidence of *Coxiella burnetii* infection in both livestock and humans in the Bragança district

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Q Fever is a zoonotic disease caused by *Coxiella burnetii*, often underdiagnosed, with a growing number of human cases reported throughout Europe. This study aimed to assess the awareness of livestock veterinarians regarding the occurrence of Q Fever in animals in the Bragança district, Northern Portugal, where four human cases have been reported since 2021. A methodological approach based on structured telephone interviews was conducted with livestock veterinarians practicing in the region for at least three years. A standardized script comprising four key questions was used, which was subsequently analyzed through thematic analysis. Of the 26 eligible veterinary practitioners, 23 (88.5%) participated. When asked whether they were aware of *Coxiella burnetii* positive cases in animals, 47.8% (11/23) responded affirmatively. Additionally, 43.5% (10/23) reported having diagnosed Q Fever or obtained laboratory confirmation of *Coxiella burnetii* in livestock. All these veterinarians observed clinical signs suggestive of the disease: six reported abortions as the only sign, three noted abortion and fever, and one mentioned abortion accompanied by mortality in young animals. This study provides the first documented evidence of *Coxiella burnetii* infections in both livestock and humans in the Bragança district and, by providing clinical and laboratory evidence of Q fever in both animals and humans in the same epidemiological context, these findings emphasize the potential under-reporting or subclinical nature of animal infection, highlighting the value of integrating serology, clinical case detection, and human surveillance within a One Health framework, underscoring the need for official reporting to enhance awareness, surveillance, and control measures implementation.

Keywords

Q fever; Coxiella burnetii; awareness.

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Toxoplasma gondii in Northeastern Portugal: A Narrative Review of a Neglected Zoonotic Infection in a One Health Context

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Toxoplasmosis is a zoonosis caused by *Toxoplasma gondii*, a protozoan parasite that infects warm-blooded animals, including humans[1]. Despite its global distribution and considerable impact on public health, animal production, and the environment, it remains overlooked, exemplifying a One Health issue[2,3]. Transmission occurs mainly through ingestion of infective stages: sporulated oocysts, via contaminated water, soil, or food, tissue cysts in undercooked meat, or tachyzoites, through transplacental transmission or unpasteurized dairy products[1,4]. Though often asymptomatic, toxoplasmosis can cause severe outcomes in immunocompromised individuals and during pregnancy. Small ruminants are particularly susceptible, with infection linked to reproductive losses and zoonotic transmission via meat[5]. In Portugal,

T. gondii infection, and toxoplasmosis, remains underreported and poorly studied. The Northeastern region (Trás-os-Montes) is especially relevant due to the high density of small ruminant farming and close human, animal and environment interactions[6]. A narrative review of the literature on the epidemiological status of *T. gondii* infection in Northeast Portugal was carried out. Sources included PubMed, Scopus, Web of Science, Google Scholar, and institutional reports (EFSA, ECDC, INE). The EFSA/ECDC/2023[7] report a 4.2% rise in congenital toxoplasmosis and a 29% positivity in small ruminants, the highest in five years. In Portugal, according to the only national survey (1979–1980)[8], seroprevalence was 47%, with the North presenting the highest percentage (51%). Subsequent studies revealed regional fluctuations[8–12] and more recently, a 72.8% value was reported amongst workers with occupational exposure[13]. In the Northeastern region (2004–2010)[14–17], active circulation of *T. gondii* was demonstrated in various animal species including wild and livestock, particularly small ruminants, as well as in women of childbearing age. The detection of genotype II, associated with moderate virulence, in meat intended for consumption reinforces the zoonotic risk[18]. These findings highlight the urgent need for updated and integrated epidemiological studies, supported by a One Health approach.

Key words

Northeastern Portugal, One Health, Toxoplasma, Zoonosis

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Q Fever in Portugal: A One Health-Oriented Literature Review

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Q fever is a zoonosis caused by *Coxiella burnetii (C. burnetii)*, a highly infectious and environmentally persistent bacterium. Its transmission from animals to humans occurs mainly via inhalation of contaminated aerosols, often originating from small ruminants. Although the disease is widely distributed in Europe, its burden remains underestimated due to nonspecific clinical presentation and insufficient surveillance integration. As part of an ongoing PhD project in Biomedical Sciences, a comprehensive literature review was conducted to assess the current state of knowledge on Q fever in Portugal. This work aimed to synthesize available data from animal, human and environmental studies, while comparing findings with the broader European context. Over 40 national studies were analyzed, covering humans, domestic and wild animals, ticks, and environmental matrices. Reported seroprevalence values ranged widely: from 0% up to 45.9% in some livestock studies, with values often exceeding 30% in goats and sheep. In human studies with occupational exposure, positivity rates reached 30.7%. Molecular detection of *C. burnetii* was reported in milk, placentas, and reproductive tissues, and in urban tick populations. The analysis revealed major gaps in national surveillance. Notably, there are no published studies specifically targeting the northeastern region of Portugal, despite its relevance in small ruminant production, and furthermore, no study to date has simultaneously assessed humans, animals and environmental matrices in the same geographic context, a key limitation for understanding transmission dynamics and implementing the One Health approach. These findings underline the need for targeted and integrated research in underrepresented regions. The next stages of the doctoral project will involve the collection and analysis of new biological and environmental samples in northeastern region of Portugal, with the goal of mapping local *C. burnetii* circulation and proposing context-adapted surveillance strategies.

Keywords

Q fever; Coxiella burnetii; One Health; Portugal

Surveillance of Zoonotic Pathogens in Bat-Associated Ticks in Portugal

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In a changing world marked by increasing environmental stressors and closer contact between humans and wild animals, it is important to survey the presence and potential spread of zoonotic pathogens carried by ticks parasitizing wild animals. Bats are suitable hosts for blood ingestion by ticks, as they lack dense fur and have large blood vessels just below the dermis. Some studies have shown that certain species of ticks that parasitize bats can also parasitize humans, and various microorganisms have already been identified in these ticks. During 2024 and 2025, ticks were collected from bat roosts, natural caves and bat boxes, and bats caught by hand in central and north Portugal. Bat boxes were primarily occupied by *Pipistrellus kuhlii*, with occasional presence of *Pipistrellus pygmaeus*, and *Nyctalus leisleri,* while caves were mostly used by *Miniopterus schreibersii*, and occasionally by *Myotis myotis*. The ticks found in bat boxes were morphologically identified as *Argas vespertilionis* (N = 168). Ticks found in bats inhabiting caves were *Ixodes vespertilionis* (N = 4), but these were all, except one, collected directly from the bat host (*M. schreibersii*). Ticks (N = 49) were tested molecularly for the presence *B.burgdorferi* s.l., relapsing-fever group *Borrelia*, and *Francisella* spp. Only one tick (2%) tested positive for *Borrelia garinii* using a nested PCR targeting the *flaB* gene. No infections with relapsing-fever *Borrelia* or *Francisella* spp. were detected. Although pathogen prevalence was low, these findings highlight the importance of continued surveillance to better understand the diversity and the potential transmission of tick-borne pathogens associated with bats.

Keywords

Ticks, Bats, Tick-borne pathogens

Knowledge of livestock producers on Q fever, brucellosis and echinococcosis and association with sociodemographic and production-related factors, in Northern Portugal

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In One Health context, this study aimed to assess livestock producers' knowledge of three important zoonotic diseases, Q fever, brucellosis (human and animal) and echinococcosis, and explore their association with sociodemographic and production-related factors, in Northern Portugal, with a central economic and social livestock activity. A cross-sectional survey was conducted with 82 livestock producers across multiple municipalities in Northern Portugal. Data were collected through structured interviews, addressing disease awareness, education level, production system typology (intensive vs. extensive), animal species reared, gender, age group, and producer's training in animal production. Descriptive statistics were used to characterize the population, and Pearson's Chi-square tests were applied to assess associations between knowledge levels and the independent variables. The results revealed substantial discrepancies in disease awareness. While knowledge of brucellosis (human: 90.2%; animal: 93.9%) was widespread, awareness of Q fever (12.2%) and echinococcosis (7.3%) was critically low. Statistically significant associations were observed between Q fever knowledge and educational attainment ($p = 0.0193$), and between human brucellosis knowledge and municipality ($p = 0.0339$). Animal brucellosis knowledge also showed a significant association with municipality ($p = 0.0173$). For echinococcosis, knowledge levels were significantly associated with animal species reared ($p = 0.0323$), municipality ($p = 0.0096$), and type of production system ($p = 0.0056$). No significant associations were observed between disease knowledge and variables such as gender or specific training in animal production. These findings highlight critical knowledge gaps at the livestock-human interface, particularly regarding neglected zoonoses. The low awareness among producers, especially those in extensive production systems or with lower educational levels, underlines the urgent need for integrative, cross-sectoral health education strategies. This study reinforces the importance of a One Health approach in zoonotic disease prevention. Promoting knowledge dissemination and surveillance through One Health-oriented programs is essential to mitigate zoonotic threats in rural communities.

Keywords

Q fever, brucellosis, echinococcosis

Acknowledgements

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ESKAPE Bacteria and Zoonotic Potential: An Emerging Concern?

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Antimicrobial resistance (AMR) is one of the greatest threats to global health, and the ESKAPE pathogens: *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp. stand at the center of this crisis. These bacteria are notorious for their ability to "escape" the action of antimicrobials and cause persistent, often untreatable infections, particularly in hospital settings[1]. While classically associated with nosocomial infections, increasing evidence suggests that some ESKAPE pathogens also have a zoonotic dimension, linking human, animal, and environmental health. *S. aureus*, including livestock-associated MRSA, exemplifies the bidirectional transmission between humans and animals, particularly pigs, poultry, and cattle[2,3]. Similarly, *E. faecium* resistant strains have been identified in food-producing animals, raising concerns about their entry into the food chain[4]. *K. pneumoniae* has shown shared clones between humans and animals, suggesting potential for cross-species dissemination[5]. For other members of the group: *A. baumannii*, *P. aeruginosa*, and *Enterobacter* spp., the zoonotic relevance is less well defined, yet their environmental persistence and presence in animals cannot be ignored. Together, these findings raise critical questions about the role of food, animal reservoirs, and environmental contamination in the global spread of AMR. The public health implications are severe: therapeutic options are increasingly limited, infections are harder to treat, and the risk of transmission across species and borders is ever-present. Preventive strategies must therefore extend beyond hospital walls, incorporating biosecurity in animal production, rational antibiotic use in veterinary and human medicine, and integrated surveillance systems. Within the One Health framework, tackling ESKAPE pathogens requires a coordinated approach that bridges gaps in knowledge, monitors zoonotic risks, and fosters collaboration across disciplines. Only through such integrated efforts can this threat to human and animal health be effectively contained.

Keywords

ESKAPE, animals, One Health

Acknowledgements

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A new approach for the development of a vaccine against African swine fever: site-directed mutagenesis for functional impairment and antigen suppression of the viral E2 ubiquitin-conjugating enzyme pI215L

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African swine fever (ASF) is an infectious viral disease that affects domestic pigs and wild boars, with high mortality and transmission capacity but absent of any treatment, representing a serious threat to the farming industry[1]. pI215L is an ubiquitin-conjugating (E2) enzyme encoded by the virus, previously suggested to have an essential role in the virus infection cycle[2]. Genetic manipulation of this protein could then be a suitable path for the design of a live attenuated vaccine against the ASF virus, which could also have the capacity to differentiate disease infection from vaccine immunization (DIVA)[1]. We began with the production of twelve recombinant pI215L containing single-point mutations in key regions, then assessing its decreased capacity to receive ubiquitin compared to the wildtype protein. We selected two mutants as basis for the design of seven multi-point mutants, encompassing the 11-15 and/or 130-134 region, which were then found to be capable of a 50 to 90% decrease of E2 activity. For the identification of immunogenic regions within the pI215L sequence, we designed two pI215L peptide libraries (25 and 9-residue length) which were incubated with sera from ASFV-infected domestic pigs. We found that the pI215L 61-69 region presents high affinity to ASF-related IgG and IgM, and the design of a third 9-residue library allowed the identification of two mutations which are able to suppress epitope recognition. These epitope mutations were then combined with previously selected functional multi-residue mutants in newly produced recombinant pI215L to access the preservation of decreased E2 activity. All in all, our work showed that site-directed mutagenesis of pI215L can be an effective method to achieve partial to total functional impairment and epitope suppression, and such mutations may be incorporated into an isolate of the ASF virus for production of a live attenuated vaccine with a DIVA feature.

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Keywords

African swine fever, pI215L, live attenuated vaccine, DIVA

Acknowledgements

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Listeria monocytogenes Meningitis in an Elderly Cancer Patient: A One Health Perspective on Foodborne Zoonoses

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Listeria monocytogenes is a Gram-positive, facultative anaerobic bacterium whose infection is primarily transmitted through contaminated food. Listeriosis, caused by this bacterium, particularly affects vulnerable groups such as immunocompromised individuals, the elderly, pregnant women, and newborns, and can progress to severe forms like meningitis, septicemia, and brain abscesses. In the One Health approach, collaboration between human, animal, and environmental health is emphasized to control its spread. Integrated surveillance and hygiene practices are essential to ensure food safety and protect public health. The objective is to evaluate the significance of listeriosis in immunocompromised patients and the need for proper care and treatment to prevent complications and transmission. The clinical case refers to a 75-year-old male patient with a history of large cell neuroendocrine carcinoma of the colon, who underwent surgery and is receiving chemotherapy (cisplatin + etoposide) for hepatic recurrence. He recently had COVID-19. The patient presented to the Emergency Department with high fever (up to 40ºC), severe prostration, anorexia, and watery rhinorrhea, without an infectious focus. He is in a febrile neutropenia context, immunocompromised, and under follow-up at the IPO. Laboratory examination showed thrombocytopenia and renal function alterations, with elevated creatinine and urea. There was a significant increase in GGT and C-reactive protein, indicating inflammation and liver impairment. Cerebrospinal fluid analysis revealed elevated proteins, normal glucose, and pleocytosis with predominance of mononuclear cells. Blood cultures confirmed *Listeria monocytogenes*, with susceptibility to ampicillin, benzylpenicillin, erythromycin, and cotrimoxazole. This case highlights the importance of early diagnosis and appropriate antimicrobial therapy, especially in oncological and immunocompromised patients. Furthermore, it underscores the need for preventive measures, such as food control and health education, to avoid infections. From the One Health perspective, it reinforces the importance of epidemiological surveillance, food safety, and multidisciplinary strategies to prevent listeriosis transmission and protect at-risk populations.

Keyword

Listeria monocytogenes; Colon cancer; Foodborne Zoonoses

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From Poultry to People: The Zoonotic Burden of *Salmonella* spp.

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Salmonellosis is one of the most significant zoonotic diseases worldwide, caused by bacteria of the genus *Salmonella*, which includes over 2500 serotypes. These Gram-negative, facultatively anaerobic bacteria are highly adaptable, able to survive in diverse environments and colonize a wide range of animal hosts. A key feature of *Salmonella* spp. is its widespread presence in domesticated animals, particularly poultry, which serve as important reservoirs for human infection. Human exposure occurs primarily through consumption of contaminated food products derived from these animals, such as meat and eggs, or through direct contact with infected birds[1-2]. Salmonellosis is the second most reported gastrointestinal infection in the EU/EEA and a significant cause of food-borne outbreaks, with 65,967 laboratory-confirmed cases reported in 2022[3]. Poultry-associated outbreaks have been reported in multiple countries, highlighting the global public health relevance of this transmission route. Human infections can vary from mild gastroenteritis to severe systemic disease, especially in young children, the elderly, and immunocompromised individuals. Complicating management, the emergence of antimicrobial-resistant *Salmonella* spp. strains has significantly limited treatment options, representing a growing challenge for public health authorities. Preventive measures remain the most effective approach, including biosecurity in poultry production, thorough cooking of poultry products, good hygiene practices, and public awareness of safe handling[4,5]. Within the One Health framework, salmonellosis exemplifies the interconnectedness of human, animal, and environmental health. Integrated surveillance, research, and coordinated interventions are essential to mitigate the risk of outbreaks, reduce the spread of resistant strains, and protect global health. While poultry represents a critical source of infection and requires targeted control measures, broader strategies addressing multiple reservoirs and the entire food chain are necessary to effectively prevent salmonellosis and ensure safer food systems worldwide.

Keywords

Salmonella spp., poultry, global health

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Zoonotic Tuberculosis: A Persistent Threat to Public Health

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Zoonotic tuberculosis (zTB), primarily caused by *Mycobacterium bovis*, represents a serious public health concern with significant implications for both human and animal health. *M. bovis* can infect a wide range of hosts, including cattle, wildlife, and humans, illustrating the complex dynamics of zoonotic transmission. Human infection often occurs through direct contact with infected animals or consumption of unpasteurized animal products[1]. Outcomes are particularly severe because *M. bovis* is intrinsically resistant to pyrazinamide, one of the cornerstone drugs in standard tuberculosis therapy. This natural resistance leaves fewer therapeutic options, and the emergence of additional antimicrobial resistance represents a further obstacle to effective treatment, amplifying the global health burden [2-3]. Moreover, diagnostic procedures for zTB remain time-consuming and technically demanding, frequently leading to delays in case detection. Such delays compromise timely initiation of therapy and worsen the prognosis for affected patients, further complicating disease control[4]. While estimates of zTB incidence and mortality remain imprecise, it is believed that approximately 140,000 new cases and 11,400 deaths occur annually worldwide. Globally, zTB accounts for around 1.4% of all tuberculosis cases, though this figure is likely underestimated. Bovine tuberculosis (bTB) is endemic in several parts of the world. Data from 119 countries indicate that *M. bovis* circulates in 59% of national cattle herds, yet only 10% of these countries have implemented zTB-specific surveillance. The absence of national bTB control programs, particularly those involving test-and-slaughter policies, further increases the risk of zoonotic transmission to humans[5]. Preventive measures are therefore critical and rely heavily on control programs in livestock, pasteurization of dairy products, and surveillance at the human–animal interface. Within the One Health framework, zTB highlights the interdependence of human, animal, and environmental health, emphasizing the need for integrated strategies to reduce transmission, protect public health, and safeguard food security.

Keywords

Zoonotic tuberculosis, antimicrobial resistance, diagnostic

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Clinical characteristics, diagnosis, and therapeutic challenges of human brucellosis: insights from recent clinical studies

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Brucellosis is a zoonotic infection caused by bacteria of the genus *Brucella*, typically transmitted through direct or indirect contact with infected livestock such as cattle and swine or by the consumption of unpasteurized dairy products. The clinical presentation in humans is usually insidious and nonspecific, with fever reported in approximately 78% of cases, along with sweating, fatigue, myalgia, arthralgia, back pain, and weight loss. Osteoarticular involvement is frequent, and nonspecific gastrointestinal manifestations are also common[1,2]. Systematic reviews indicate that severe complications such as endocarditis and neurobrucellosis occur in about 1% and 4% of patients, respectively, while up to 10% of men may develop epididymo-orchitis[3]. Large clinical series with more than 1,500 patients have demonstrated diverse systemic laboratory alterations, including reduced fibrinogen, total protein, and albumin levels, as well as impaired hepatic and renal function, reflecting the multisystemic impact of the disease[4]. The diagnosis of brucellosis remains challenging due to the absence of pathognomonic signs. Serological assays such as the Rose Bengal test and IgG/IgM ELISA are widely used, while molecular methods like PCR have shown improved sensitivity and specificity, although results vary across different studies and laboratory settings. Bacterial culture is still regarded as the gold standard, but its sensitivity is limited and incubation times are long[5]. Treatment is based on combined antibiotic regimens. A meta-analysis of randomized controlled trials demonstrated that doxycycline combined with rifampicin is associated with significantly higher rates of therapeutic failure and relapse compared with doxycycline plus streptomycin, while more recent systematic reviews highlight the effectiveness of doxycycline combined with gentamicin or triple therapy regimens as more reliable alternatives[6]. Altogether, these findings confirm that human brucellosis is a heterogeneous and systemic disease, posing substantial diagnostic and therapeutic challenges, and emphasize the need for improved diagnostic capacity, integrated One Health surveillance, and optimized therapeutic strategies to reduce its burden, particularly in endemic regions.

Keywords

Clinical manifestations; diagnosis; antibiotic therapy; zoonosis

Acknowledgements

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Antimicrobial Resistance Profiles of *Klebsiella spp.* Isolated from Healthy Chickens Intended for Consumption in Portugal

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Clinical infections caused by multidrug resistant (MDR) bacteria represent a major global health concern. *Klebsiella spp.*, particularly K. *pneumoniae*, can acquire resistant to a broad range of antimicrobials through the production of enzymes that inactivate multiple antibiotic classes, including aminoglycosides, fluoroquinolones, and especially beta-lactams, thereby severely limiting treatment options. Considering the importance of Klebsiella spp. in both veterinary and human medicine, surveillance studies are needed to assess antimicrobial susceptibility profiles and to evaluate poultry meat as potential reservoir. Forty-three *Klebsiella spp.* strains were isolate from healthy chickens intended for human consumption. Antimicrobial susceptibility testing against 14 antibiotics (penicillins, cephalosporins, carbapenems, beta-lactams, fluoroquinolones, aminoglycosides, tetracyclines, and others) was performed by agar disck diffusion according to EUCAST and CLSI guidelines. Among the isolates, 46.5% were classified as MDR. High resistance rates were observed for penicillins (95%), fluoroquinolones (53.5%), and trimethoprim-sulfamethoxazole (51%). Moderate resistance was detected to tetracyclines (20.9%) and aminoglycosides (11.6%), while lower levels were observed for phenicols (4,7%). No resistance was detected to carbapenems, monobactams, or cephalosporins. Nearly half of the *Klebsiella spp.* isolates from chickens demonstrated multidrug resistance, underscoring the poultry industry as a potential reservoir of resistant strains with implications for food safety and public health. Ongoing studies are focused on investigating the prevalence of antimicrobial resistance and virulence genes in these isolates.

Keywords

Chickens, One Health, K. pneumoniae, Antimicrobial Resistance

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Antimicrobial Resistance and Zoonotic Potential of *Staphylococcus aureus* from Rabbits in Northern Portugal

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Staphylococcus aureus is a clinically important pathogen in both human and veterinary medicine. Rabbits, as livestock species and companion animals, may act as reservoirs of antimicrobial resistant strains, raising concerns within the One Health framework. This study aimed to investigate the antimicrobial resistance profiles and molecular characteristics of S. *aureus* isolates from rabbits and their farm workers. A total of 152 samples were collected from healthy and diseased rabbits from farms in northern Portugal, together with 19 samples from farm workers. S. *aureus* isolates were identified by biochemical and molecular methods. Antimicrobial susceptibility testing against 14 antibiotics was performed using the Kirby-Bauer method following EUCAST and CLSI guidelines. Molecular analyses included detection of resistance genes, *agr* typing, and SCC*mec* characterization of MRSA isolates. Fifty-five S. *aureus* isolates were identified, of which 30.9% were MRSA and 69.1% MSSA. Isolates originated primarily from mastitis (34.6%), pododermatitis (30.9%), healthy rabbits (30.9%), and abscesses (3.6%), while none were detected in workers. High resistance rates were observed to ciprofloxacin (80%), penicillin (70.9%), and clindamycin (63.6%), with lower levels to tetracycline, erythromycin, and aminoglycosides. All isolates remained susceptible to linezolid. The *mec*A gene was consistently detected, most isolates belonged to *agr* type III, and SCC*mec* type III was predominant among MRSA strains. The presence of multidrug resistant MRSA in rabbits highlights the potential role as reservoirs of clinically relevant S. *aureus*. Although no human isolates were identified, these findings emphasize the importance of ongoing surveillance and prudent antimicrobial use to mitigate zoonotic risks in rabbit farming.

Keywords

Rabbit, One Health, MRSA, Antimicrobial Resistance

Acknowledgements

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The Overlooked Role of Rabbits in Antimicrobial Resistance and Zoonotic Risk

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Antimicrobial resistance (AMR) is a critical global health challenge, traditionally studies in major food-producing animals such as poultry, swine and cattle. However, rabbits, widely used for meat production, as companion animals, and in biomedical research, remain an underexplored host regarding their role in the emergence and dissemination of AMR. This review aims to synthesize current knowledge on AMR in rabbits and discuss their overlooked contribution to the One Health framework. A review of studies published in the last two decades was performed, focusing on bacterial pathogens isolated from rabbits, patterns of antimicrobial resistance, and reported resistance genes. Emphasis was placed on food safety, zoonotic transmission, and public health implications. Available evidence indicates frequent detection of multidrug resistant *Escherichia coli* and *Staphylococcus spp.* in rabbits, including methicillin resistant strains and extended-spectrum beta-lactamase (ESBL) producers. Despite this, systematic surveillance data are scare, particularly when compared to other food producing animals. The close contact of rabbits with humans, either as pet or through the food chain, underscores their potential as reservoirs and vectors of AMR. Rabbits represent a ne lected but relevant niche within the AMR landscape. Their inclusion in surveillance systems is urgently needed to strengthen One Health strategies and better understand the dynamics of resistance transmission across species.

Keywords

Rabbit, One Health, Zoonoses, Antimicrobial Resistance

Exploring the gene expression of canine monocyte-derived cells following three differentiation cocktails

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Dendritic cells are specialized in antigen presentation to naïve T-cells, bridging innate and adaptive immunity, thus being studied as immune-precision tools against diseases such as Leishmaniosis[1]. Monocyte-derived cells (MDCs), including dendritic cells and macrophages, can be obtained from peripheral blood mononuclear cells (PBMCs), using appropriate cytokine cocktails. The characterization of MDCs represents a challenge with gaps to fulfil, especially in dog[2]. This work aimed to characterize the gene expression of MDCs, comparing three distinct protocols for cell differentiation. PBMCs were isolated from blood of ten healthy dogs and differentiated with: P1-cell culture medium (CCM); P2-CCM and colony stimulation factor (CSF); P3-CCM, CSF and interleukin-4. At day 4, half medium was renewed, and at day 7, whole medium was changed, with CCM only, or with phorbol-12-myristate-13-acetate (PMA), and cells were incubated for 24 h. At day 8, cells were collected and the gene expression of Pattern Recognition Receptors (NOD1, NOD2, TLR2 and TLR4), cytokines (IL-10, IL-12p40, TGF-β and TNF-α) and co-stimulatory (CD80) molecules was determined by reverse transcription real-time PCR (RT-qPCR). Statistical analysis was conducted with the Friedman Test and Wilcoxon Signed-Rank Test to assess the effect of the different protocols and PMA, respectively. P3-differentiated cells were the most responsive to PMA, presenting a significant downregulation of CD80, IL-10, NOD1, TLR2 and TLR4, and an upregulation of TNF-α. TNF-α was increased upon PMA stimulation in cells derived from all three protocols. NOD1 was significantly downregulated in P3, compared with P1, while TGF—β significantly increased from P2 to P3, upon addition of IL-4 during differentiation. Protocol P3 resulted in the differentiation into cells more sensitive to PMA stimulation, responding through a mixed profile including both anti-inflammatory/regulatory and proinflammatory features. These findings contributed to understanding the characterization of MDCs, enhancing their complexity and their potential to be further explored in immunological research studies.

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Keywords

Monocyte-derived dendritic cells, RT-qPCR, innate receptors, cytokines.

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Recent Advances in *Leptospira* spp. Zoonosis: Epidemiology, vaccine Efficacy, and clinical implications

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Leptospira spp. remains a major zoonotic pathogen with global distribution and significant impact on both public and animal health. Recent studies have provided updated insights into prevalence, vaccine efficacy, and clinical manifestations. Epidemiological surveys confirm a broad host range and regional variability. A One Health study in Jordan revealed a human seroprevalence of 4.0% (95% CI: 2.5–6.1)[1], while a global meta-analysis in goats estimated a prevalence of 18.6% (95% CI: 15.1–22.7), with the highest values in North America (34%) and lower in Europe (12%)[2]. In Chile, dairy cattle showed a within-herd prevalence of 5.9% (95% CI: 4.9–6.8) and herd-level prevalence of 42.2%, with *L. borgpetersenii* serovars Hardjo and Tarassovi, and *L. interrogans* Pomona being the most frequent[3]. Vaccination remains a key preventive measure. Recent canine studies indicate 84% protection against clinical disease and 88% against renal carrier state[4]. In cattle, vaccines targeting the Hardjo serovar showed an efficacy of 88.7% (95% CI: 81.0– 93.2%) in preventing urinary shedding[5]. Clinically, human leptospirosis presents with a wide spectrum from mild flu- like illness to severe complications, including renal and hepatic failure, pulmonary hemorrhage, and meningitis. A systematic review of leptospirosis-associated acute pancreatitis reported a 22.7% mortality among 79 cases[6]. In livestock, the disease contributes to reproductive losses and economic burden. Despite advances, leptospirosis remains a neglected but persistent zoonotic threat. Strengthening surveillance, integrated vaccination programs, reservoir control, and early diagnostic capacity are crucial[7]. A One Health perspective, combining human, veterinary, and environmental interventions, is essential to reduce morbidity and mortality linked to *Leptospira spp.* infections.

Keywords

Leptospira spp.; zoonosis; vaccine efficacy; One Health

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Intestinal infection with *Prototheca bovis* in a dog with hemorrhagic colitis from the south of Portugal.

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Protothecosis is an infectious disease caused by a unicellular algae of the genus Prototheca. Cutaneous, subcutaneous and/or systemic forms are described worldwide in humans and animals. In animals, the most prevalent form of protothecosis is bovine mastitis resulting in acute infections (granulomatous mastitis) or with chronic progression. Until now, in Portugal, protothecosis due to *P. bovis*, *P. blaschkeae* and *P. cifferri* has only been described in cattle but not in other animals or humans. *P. wickerhamii* is an important human pathogen, followed by *P. bovis*, *P. cutis,* and *P. miyajii*. We report a case of intestinal protothecosis in an 8-year-old Rhodesian Ridgeback dog presenting with chronic hemorrhagic diarrhea, weight loss, and intermittent anorexia, refractory to treatments. A dermatitis not compatible with a Prototheca infection also affected the dog four months prior, with no other body systems being impacted. The dog had access to the outdoors and used to play in a puddle with stagnant water. Diagnosis was based on histopathology from colonic endoscopic biopsies and confirmed by molecular investigations (PCR and sequencing). Microscopically, the colonic tissue showed an eosinophilic, granulomatous and lymphoplasmacytic colitis associated with intralesional algae with sporangia and morula-like shaped organisms that stained positive for PAS (periodic acid Schiff) and Grocott stains, consistent with Prototheca species. Mithocondrial *Cyt b* sequence confirmed *P. bovis* infection. Following diagnosis, the dog received treatment with amphotericin B and itraconazole, combined with colonic lavage. There has been alternating periods of clinical improvement and relapse up to now, but no signs of disseminated disease. Although a rare disease, protothecosis in dogs represents a life-threatening disease. Dogs typically develop disseminated form that usually begin with chronic bloody diarrhea followed by typical ocular and neurological signs such as ataxia, blindness, deafness, or seizures. *Prototheca bovis* formerly know has *P. Zopfii* genotype 2 is a zoonotic agent and has not been previously reported in Portugal affecting dogs. Because these algae are ubiquitous in the environment, from a public health perspective, dogs could serve as indicators of algal presence in the environments they frequently share with humans.

Keywords

Prototheca bovis, dog, colitis, Portugal

Tick-borne Pathogens in Companion Animals in Portugal: A One Health Surveillance Approach

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This study aimed to assess the emergence and re-emergence of tick-borne diseases (TBDs) in Portugal by correlating hemoparasite infection in companion animals with vector-borne disease dynamics, applying a One Health perspective. Between 2015 and 2024, a total of 1,169 clinically suspected animals treated at the Hospital Veterinário de Santarém (HVS) underwent serological testing for *Rickettsia conorii*, *Babesia canis*, *Ehrlichia* spp., and *Haemobartonella* spp. A total of 3,791 serological tests were performed (mean: 3.2 per animal), with 437 animals testing positive for at least one hemoparasite. Between 2020 and 2024, the number of tests nearly tripled, increasing from 894 to 2,883, while the overall positivity rate rose from 29.5% to 39.9%. Notably, cases of rickettsiosis and hemobartonellosis increased significantly, indicating enhanced circulation of their respective vectors. In parallel, a national vector surveillance initiative identified *Hyalomma* spp., *Rhipicephalus sanguineus*, *Ixodes ricinus*, and *Dermacentor* sp. as the main tick species involved in the transmission of these hemoparasites, as well as zoonotic agents such as Crimean-Congo Hemorrhagic Fever Virus (CCHFV) and tick-borne encephalitis virus (TBEV). The high prevalence of seropositive animals at HVS reflects the growing exposure risk posed by ticks in domestic environments and underscores the importance of integrating veterinary data into broader public health surveillance. These findings highlight the role of companion animals as effective sentinels for early detection of TBDs, reinforcing the relevance of integrated, cross- sectoral strategies for zoonotic disease monitoring and prevention within a One Health framework.

Keywords

tick-borne diseases; hemoparasites; companion animals; vectors; zoonoses; One Health; Portugal

The interactive dynamics of *Pseudomonas aeruginosa* in global ecology

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Pseudomonas aeruginosa is an opportunistic pathogen with remarkable ecological versatility, colonizing a wide range of natural, clinical, and anthropogenic environments. Its ability to persist in water, soil, plants, animals, and humans is linked to minimal nutritional requirements, adaptability to stress conditions, and the capacity to form biofilms. In healthcare settings, *P. aeruginosa* represents a leading cause of hospital-acquired infections, particularly in intensive care units, where multidrug-resistant (MDR) and extensively drug-resistant (XDR) clones are prevalent. The bacterium's resistance is driven by multiple mechanisms, including β-lactamases, efflux pumps, and high-risk clonal dissemination. Beyond hospitals, environmental reservoirs such as wastewater and agricultural ecosystems contribute to the spread of resistance genes, illustrating the interconnectedness of clinical and natural niches. Its dynamic interactions with other microorganisms, involving competition, commensalism, and cooperation, further shape microbial communities and highlight its role in the One Health framework. This work emphasizes the need for integrated surveillance and sustainable strategies to mitigate the global threat of antimicrobial resistance associated with *P. aeruginosa*.

Keywords

Pseudomonas aeruginosa; antimicrobial resistance; microbial ecology; One Health

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Wildlife and aquatic reservoirs of *mec*C-MRSA: a study on prevalence, genetic diversity, and resistance

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The *mec*C gene in methicillin-resistant *Staphylococcus aureus* (MRSA) is an emerging determinant of resistance in wildlife and environmental settings, with implications for antimicrobial resistance ecology and zoonotic risk. This study investigates *mec*C-positive MRSA from wild rodents, owls, hedgehogs, and surface waters, assessing their prevalence, genetic diversity, and resistance profiles. Samples included hedgehogs (n=110), rodents (n=204), owls (n=114), and surface waters (n=78). Cultures were processed using BHI broth with 6.5% NaCl, followed by selective plating, and isolates were characterized by whole-genome sequencing (STs, spa types, and resistance genes). All *mecC*-MRSA isolates displayed resistance to penicillin, mediated by the *blaZ*-*SCCmec*XI gene, but remained susceptible to non-β-lactam antibiotics. Among 204 rodent samples, 38 (18.6%) *S. aureus* isolates were identified, of which three were *mecC*-MRSA belonging to ST1945/CC130-t1535, *agr* type III, and SCC*mec* XI. These isolates carried IEC type E genes, potentially indicating a human origin, although their presence in wildlife raises questions about zoonotic potential. In 114 owl samples, 23 (20.2%) *S. aureus* isolates were identified, including one *mecC*-MRSA assigned to ST1245/CC130-t843. This isolate lacked IEC genes but harbored the *etd2* gene. Hedgehog samples yielded 22 (20%) *S. aureus* isolates of which three were *mecC*-MRSA. Two belonged to ST130-t842, and one to ST49 with a novel *spa* type, showcasing host-specific diversity. From 78 surface water samples, 33 (42.3%) *S. aureus* isolates were recovered, including four *mecC*-MRSA representing ST425-t742 and ST30-t843, highlighting the genetic diversity of *mecC*-positive strains in aquatic environments. The genetic heterogeneity of *mecC*-MRSA among wildlife and environmental sources highlights their significant role in the AMR landscape. The diversity of clonal lineages across different ecological niches suggests an adaptive potential of *S. aureus* to thrive in varied hosts and environments. These findings emphasize the importance of monitoring these reservoirs to mitigate potential zoonotic risks and understand the spread of resistance.

Keywords

Listeria monocytogenes; Chicken by-products; Antimicrobial resistance; Food safety

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Multidrug-resistant *Enterobacteriaceae* in coastal environments: evidence from Portuguese beaches and implications for zoonotic transmission within a One Health framework

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Coastal environments, shaped by human activity and wildlife, are important reservoirs for antimicrobial-resistant bacteria with zoonotic potential. Understanding their distribution in these ecosystems is essential within a One Health framework. This study investigated *Enterobacteriaceae* isolated from Portuguese beaches, characterized their antimicrobial resistance, and explored resistance genes by whole genome sequencing (WGS).

From October 2021 to October 2022, 265 environmental samples (dry gull feces, sand, seawater, stagnant water) were collected from Berlenga Grande, Aver-o-Mar, Matosinhos, and Miramar. Enterobacteriaceae were identified on Chromocult agar, tested for antimicrobial susceptibility (Kirby-Bauer), and subjected to WGS. A total of 42 *Enterobacteriaceae* (15.8%) were recovered, most frequently from sand (22.2%) and gull feces (17.1%). Species included *Klebsiella pneumoniae* (n=9), *Escherichia coli* (n=7), *Leclercia pneumoniae* (n=10), *Enterobacter hormaechei* (n=4), *Citrobacter braakii* (n=2), and single isolates of *Enterobacter wuhouensis*, *E. bugandensis*, *E. mori*, *E. ludwigii*, *E. kobei*, *Klebsiella michiganensis*, *K. oxytoca*, *Citrobacter gillenii*, and *Kluyvera georgiana*. Multidrug resistance was detected in the majority of *Enterobacter* and *Klebsiella* isolates, with phenotypic resistance to beta-lactams, quinolones, aminoglycosides, fosfomycin, and phenicols. WGS revealed key resistance determinants: *Enterobacter* carried *oqx*AB, *qnr*E, *bla*$_{\text{ACT}}$ variants (*bla*$_{\text{ACT-14/22/23/28/47}}$),*fos*A, and *acr*D; *Klebsiella* harbored *bla*$_{\text{SHV}}$ (*bla*$_{\text{SHV-1/11/108}}$), *bla*$_{\text{OXY-1/2}}$, *oqx*AB, *fos*A, and *acr*D; *Citrobacter* carried *bla*C$_{\text{MY}}$, *qnr*B10, and *acr*D. Multilocus sequence typing identified clinically relevant lineages: *E. hormaechei* ST574, *E. kobei* ST125, *C. braakii* ST295, *K. michiganensis* ST11, *K. oxytoca* ST199, and *K. pneumoniae* distributed across eight STs. *E. coli* isolates belonged to high-risk lineages ST48, ST58, ST372, and ST648. This study documents the presence of multidrug-resistant and clinically significant Enterobacteriaceae in coastal environments. Beaches, influenced by seabird populations and human activity, act as hotspots for the dissemination of resistance, underscoring the zoonotic risks and the importance of surveillance under the One Health paradigm.

Keywords

Antimicrobial resistance, Enterobacteriaceae, Coastal environments, One Health

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Genetic diversity and antimicrobial resistance of zoonotic *Listeria monocytogenes* from chicken by-products

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Listeria monocytogenes and other *Listeria* species are critical pathogens linked to foodborne diseases, posing serious concerns for food safety and public health. This study assessed the occurrence, antibiotic resistance profiles, and genetic diversity of Listeria spp. isolated from edible chicken by-products, including livers, hearts, necks, gizzards, and feet, obtained from supermarkets and retail markets. Out of 72 samples analyzed, 15 (20.8%) tested positive for *Listeria*. Species identification via MALDI-TOF detected 12 isolates of *L. monocytogenes*, including two distinct strains from a single sample, along with three isolates of *L. welshimeri* and one of *L. innocua*. Antibiotic susceptibility was assessed using the Kirby-Bauer method. Resistance and virulence genes, along with MLST, were determined through Whole Genome Sequencing. *L. monocytogenes* was predominantly detected in chicken necks, followed by livers, hearts, and feet. *L. welshimeri* was identified in liver, neck, and heart samples, while *L. innocua* was found in one gizzard sample. Except for a single *L. welshimeri* isolate resistant to trimethoprim-sulfamethoxazole, all other isolates were susceptible to the tested antibiotics. Lineage analysis placed *L. monocytogenes* isolates into Lineages I and II, with clonal diversity represented by ST155, ST504, ST87, ST8, ST121, ST378, and ST532. The *L. innocua* isolate was ascribed to ST532. This study highlights diverse *L. monocytogenes* lineages in chicken by-products, with potential foodborne and zoonotic risks. While most isolates were antibiotic-susceptible, the detection of resistance in one *L. welshimeri* strain reinforces the need for continued surveillance of *Listeria* in poultry products to protect public health.

Keywords

Listeria monocytogenes; Chicken by-products; Antimicrobial resistance; Food safety

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Phenolic Compounds from Cherry (*Prunus avium*) as Natural Adjuvants to Antibiotics: A Sustainable Strategy against Antimicrobial Resistance

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The alarming rise of antimicrobial resistance calls for alternative strategies capable of restoring antibiotic effectiveness while aligning with sustainability principles. Phenolic compounds extracted from cherry (*Prunus avium*) have emerged as multifunctional bioactives with antimicrobial properties. Therefore, this review synthesizes evidence on their potential as antibiotic adjuvants against resistant pathogens of human and veterinary concern. Experimental studies reveal that cherry phenolics, particularly anthocyanins, chlorogenic acid, and quercetin, can enhance antibiotic activity[1,2]. These effects have been observed in both Gram-positive and Gram-negative bacteria, including *Escherichia coli*[3], *Bacillus cereus*[4], and *Staphylococcus aureus*[2]. This bioactivity is mainly exerted through the disruption of bacterial membranes, the inhibition of biofilm formation, the modulation of efflux pump activity, and the interference with quorum-sensing communication. Moreover, authors showed that phenolics from sweet cherry by-products have very interesting antimicrobial properties[5]. The recovery of these compounds from processing residues contributes to waste reduction and supports a circular bioeconomy. By integrating such natural compounds into antimicrobial strategies, either as dietary supplements, food preservatives, or therapeutic coadjuvants, cherry phenolics could reduce antibiotic dependence and limit the spread of resistance genes along the food chain. Given their proven activity against zoonotic bacteria relevant to veterinary and human health, these compounds could contribute to improved antibiotic stewardship in both sectors, helping prevent cross-species transmission of resistance. In conclusion, *P. avium* phenolics offer a promising and sustainable approach to control resistant infections, reinforcing the interconnectedness between animal, environmental, and human health promoted by the One Health paradigm.

Keywords

Prunus avium, phenolic compounds, antibiotic synergy, antimicrobial resistance.

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Exploring Citrus-Derived Phenolic Compounds as Antibiotic Adjuvants: A One Health Approach to Combat Antimicrobial Resistance

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Antimicrobial resistance poses a global threat to human, animal, and environmental health. Within the One Health framework, there is an increasing demand for natural bioactive compounds capable of enhancing antibiotic efficacy while supporting sustainable production systems. Citrus by-products, particularly orange (*Citrus sinensis*) peels, represent a rich and underutilized source of phenolic compounds including hesperidin, and naringenin[1]. Recent studies have shown that these compounds could inhibit multidrug-resistant bacteria of zoonotic importance, such as ***Staphylococcus aureus,*** and decrease biofilm formation, with naringenin displaying higher efficacy than hesperidin[2]. Antimicrobial activity against ***Pseudomonas aeruginosa***, ***Escherichia coli***, ***Bacillus cereus***, ***Klebsiella pneumonia*** has also been reported. Furthermore, methanolic peel extracts exhibiting the strongest effects[3]. Mechanistic insights suggest that orange-derived phenolics can increase bacterial membrane permeability, inhibit efflux pumps, and downregulate genes involved in virulence and quorum-sensing systems[4]. Additionally, the antioxidant and anti-inflammatory properties of these molecules can attenuate host tissue damage during infection, complementing antimicrobial therapy[5]. The occurrence of these resistant pathogens in both humans and food-producing animals underscores the need for alternative control strategies that are safe and environmentally sustainable. Beyond their pharmacological potential, the valorization of citrus residues supports a circular economy by converting agri-food waste into high-value natural antimicrobials. Integrating such phytochemicals into therapeutic protocols or feed supplementation strategies may reduce antibiotic usage in livestock and human medicine, helping mitigate antimicrobial resistance dissemination. Overall, the exploitation of orange-derived phenolics as antibiotic adjuvants exemplifies an innovative and eco- responsible approach to antimicrobial stewardship within the One Health concept.

Keywords

Citrus sinensis, phenolic compounds, antibiotic adjuvants, One Health

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Post-Mortem Detection of Bovine Eosinophilic Myositis in Northern Portugal: Prevalence, Zoonotic Cases and Meat Inspection Outcomes

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Bovine eosinophilic myositis (BEM) is an uncommon myopathy that affects the striated muscles of cattle. Since it does not cause clinical signs in these animals (except in the acute form), most lesions are detected in slaughterhouses during post-mortem inspection, resulting in partial or total carcass condemnation depending on their severity. Between June and December 2024, during post-mortem inspection at a slaughterhouse in the Greater Porto region, and under the framework of Commission Implementing Regulation (EU) 2019/627, 96 cases of eosinophilic myositis were detected among 23,215 slaughtered cattle (0.41%). Most lesions were initially found in the masseter muscles, alerting the official veterinarian to the need for a more detailed post-mortem examination. In most cases, this led to incision of other muscles such as the tongue and diaphragm, which are usually inspected visually. The eosinophilic myositis lesions showed variable macroscopic appearances, ranging from single to multiple nodular lesions, with colours varying from yellowish-white to yellow-greenish. Occasionally, more diffuse greenish areas were also observed. After detection, lesions were collected and divided into two sampling containers: one preserved in 10% formalin for histopathological analysis and the other frozen at −18 °C for molecular testing (PCR). The aim of this investigation is to characterize BEM macroscopically and histologically, as well as to study potential etiological agents, particularly *Sarcocystis spp.* In this study, zoonotic *Sarcocystis* species were identified, representing the first confirmed cases in Portugal. These findings highlight the importance of veterinary meat inspection as an active surveillance tool for animal and public health protection, reinforcing the need for an integrative One Health approach.

Keywords

bovine eosinophilic myositis; meat inspection; carcass condemnation; Sarcocystis spp.

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Occurrence of biofilm-forming *Staphylococcus aureus* in ready-to-eat foods and food handlers' hands at university establishments

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Staphylococcus aureus is an important foodborne pathogen, frequently associated with contamination events in food products and food handlers. Its ability to form biofilms increases survival in diverse environments, which represents challenges for food safety and hygiene control. This study aimed to investigate the biofilm-forming ability of *S. aureus* isolates recovered from RTE foods and food handlers' hand samples. By comparing biofilm biomass across both sources, the objective was to better understand the potential risk of persistence and cross-contamination in the food chain. A total of 156 RTE food samples and 102 hand swabs from food handlers were collected at university establishments. Microbiological analyses were performed according to ISO 6888-3:2003. We assessed biofilm formation of *S. aureus* isolates using the standard semiquantitative microtiter plate assay at 24 h, with biofilm biomass quantified by crystal violet staining. This method was performed following the protocol described by Silva (2023)[1], with minor modifications. Biofilm production was classified as non, weak, moderate, or strong according to the optical density cut-off (ODc). Results were then normalized against *S. aureus* ATCC® 6538 to ensure consistent comparison. Overall, 44.83% of the isolates were classified as biofilm producers (44.83%), corresponding mostly at weak to moderate levels. A considerable proportion of strains displayed moderate biofilm-forming ability (20.69%), both among RTE and hands isolates, while no strain was categorized as a strong biofilm producer. On average, RTE isolates showed a biofilm biomass of 62.69 ± 14.64, whereas hand isolates exhibited a mean of 69.71 ± 36.42. No statistically significant differences were observed between food and hand isolates, regarding biomass formation ($p > 0.05$). A non-negligible biofilm-forming capacity was observed among *S. aureus* isolates, highlighting the potential risk of cross-contamination when hygiene practices are inadequate. Since RTE foods are consumed without further thermal treatment, contamination most likely occurred post-cooking, reinforcing the need for strict hygiene measures among food handlers.

Keywords

biofilms, ready-to-eat foods, Staphylococcus aureus, food handlers.

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Detection and Molecular Characterization of *Giardia* and *Cryptosporidium* in Pigs and humans in Southern Mozambique

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In Mozambique, there is currently no official information on the occurrence of *Giardia* and *Cryptosporidium* in pigs, and the available data focus on infection by *Taenia solium*, particularly in cysticercosis, in the province of Tete, in the center of the country[1,2]. Regarding molecular data, the species, genotypes, variants, and subtypes of these parasites involved in swine infections, their transmission routes, and the role that pigs play as potential reservoirs of infection for humans are unknown. For this reason, the main objective of this study is to determine the prevalence and genetic diversity of *Giardia* and *Cryptosporidium* in pigs and humans in southern Mozambique.

A cross-sectional epidemiological study was conducted between November 2024 and May 2025 in the provinces of Inhambane, Gaza, and Maputo. A total of 269 fecal samples were collected from pigs and preliminarily processed using the Ritchie centrifugation and acid-fast methods.

Eight distinct parasites were identified using parasitological methods, namely: *Giardia* spp with 13.38% (36/269), *Cryptosporidium* 23.79% (64/269), *Balantidium coli* 26.02% (70/269), *Entamoeba* spp 30.11% (81/269), Coccidia 59.85% (161/269), *Ascaris* 17.1% (46/269), strongyle type 26.77% (72/269), and *Trichuris* 5.58% (15/269). These data are preliminary and point to the circulation of several groups of parasites in pigs in southern Mozambique.

Investigations are underway to assess the prevalence of *Giardia* and *Cryptosporidium* by PCR, as well as the molecular characterization of the parasites (species, genotypes, variants, and subtypes), to identify zoonotic variants and clarify the role played by pigs in the transmission of pathogens to humans in the region.

Keywords

Epidemiology, zoonoses, pigs, and Mozambique.

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Brucellosis in Portugal: A systematic review and meta-analysis of animal and human *Brucella* spp. infections (2001–2025)

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Brucellosis remains one of the most widespread zoonoses globally, yet its true burden in Portugal remains insufficiently characterised[1,2,3,4]. This study presents the first nationwide systematic review and meta-analysis of *Brucella* spp. infections across both animal and human populations in Portugal, following PRISMA and MOOSE guidelines. A total of 871 studies were screened, yielding 62 eligible reports published between 2001 and 2025, including 6,137,223 animals and 8,724 humans tested for *Brucella* spp. using various diagnostic methods (e.g., RSAT, ELISA, IFAT, qPCR). Meta-analytical estimates based on a random-effects model revealed a pooled prevalence of 2.42% (95% CI: 1.79–3.13%) in animals and 10.57% (95% CI: 8.80–12.47%) in humans, with high heterogeneity ($I^2 > 84\%$). The overall pooled prevalence across species was 4.49% (95% CI: 3.77–5.27%), suggesting widespread and underdiagnosed exposure to *Brucella* spp., especially in occupationally exposed groups and livestock-rich regions. The asymmetry observed in the funnel plot, along with statistically significant results from Egger's ($p = 0.0004$) and Begg's ($p = 0.0077$) tests, suggests the presence of publication bias and small-study effects. These findings expose critical surveillance gaps and support the implementation of integrated One Health strategies, combining veterinary, medical, and environmental monitoring. The systematic synthesis of seroepidemiological data highlights the urgent need for harmonised diagnostic protocols, cross-sectoral data sharing, and improved awareness of *Brucella* spp. as a zoonotic threat in Portugal.

Keywords

Brucellosis, Meta-analysis, One Health, Zoonosis

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